PCR detection of toxic shock syndrome toxin of *Staphylococcus aureus* from Tripoli, Libya

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Sixty-three *Staphylococcus aureus* strains (40 from clinical sources and 23 from food sources) were examined for toxic shock syndrome toxin-1 (TSST-1) using PCR, phage typed using the international phage set (IPS) and tested for their susceptibility to antibiotics. Only three strains (all from clinical sources) were positive for the TSST-1 gene (*tst*). The majority of *S. aureus* strains that were typeable by IPS belonged to group II. Resistance to one or more antibiotics was detected in 47.5 and 73.9 % of clinical and food strains, respectively. This is the first time that PCR detection of *tst* in *S. aureus* has been reported from Libya, and further studies are needed on the occurrence of toxic shock syndrome in the community and the role of TSST-1-producing *S. aureus* in this disease in Libya.

INTRODUCTION

Staphylococcus aureus can potentially cause a wide range of infections, from relatively minor skin infections to life-threatening systemic illnesses, in both healthy and immunocompromised people. However, the ability of *S. aureus* strains to produce disease, in both clinical and food settings, depends, among other determinants, on its ability to produce extracellular toxins. A number of staphylococcal enterotoxins (SEs), classified as A, B, C1, C2, C3, D or E, can be produced by some strains (Pimbley & Patel, 1998). Most *S. aureus* strains isolated from patients with toxic shock syndrome (TSS), a severe acute illness that rapidly leads to multiorgan system failure, produce a toxin known as toxic shock syndrome toxin-1 (TSST-1). Although classically associated with tampon use, TSS has also been associated with a variety of non-menstruation-related conditions (Hertzer, 2001). Data regarding TSST-1 of *S. aureus* isolated in Libya are lacking. The present study was carried out to address such issues. In addition, isolates were tested for their susceptibility to antibiotics and were phage typed using the international phage set (IPS).

METHODS

Source and identification of *S. aureus* strains. Sixty-three *S. aureus* strains from Libya were included in the present study; 40 were from clinical sources (wound, blood, ear, eye, throat and umbilical infections) and 23 were from food sources (meat, cake, ice cream and pizza). *S. aureus* strains were grown on blood agar at

37 °C. After overnight incubation, strains were examined by Gram stain and using catalase and coagulase tests as described previously (Duguid, 1989). Isolates were also tested using the Staphytest Plus kit (Oxoid) for identification of *S. aureus*.

Detection of the TSST-1 gene. The method described previously by Johnson & Tyler (1993) was employed. One to two loopfuls of cells from test cultures on blood agar plates were used for DNA extraction after treatment with lysozyme (Sigma) and vortexing for 2 min. Relative molecular masses of the PCR products were obtained by size comparison with a 100 bp ladder marker (Pharmacia). Detection of *tst* in *S. aureus* strains was carried out using PCR as described previously (Johnson & Tyler, 1993). The sequences of the primers used were 5'-ATGGCAGCATCAGCTTGATA-3' and 5'-TTTCCAATAACCACCGTTT-3' (synthesized at the Agricultural Biotechnology Center, Gödöllő, Hungary). PCR conditions were as described previously (Johnson & Tyler, 1993). Amplification was carried out in an Applied Biosystems GeneAmp PCR system using the following steps: 1 cycle at 94 °C for 4 min followed by 30 cycles of denaturation for 2 min at 94 °C, annealing of primers for 2 min at 55 °C and primer extension for 1 min at 72 °C with autoextension. After 30 cycles, the PCR tubes were incubated at 72 °C for 7 min before cooling in the block to 4 °C. Visualization of amplified products was carried out by electrophoresis through a 2 % ultra-pure agarose gel in 1 × TBE buffer (10 × TBE is 108 g Tris, 55.5 g boric acid, 9.3 g EDTA, pH 8.3, adjusted to 1000 ml with water) and ethidium bromide staining. TSST-1-producing *S. aureus* was provided by Mr G. Kovacs (Department of Microbiology, Etövös Loránd University, Budapest) and used as a positive control in all experiments.

S. aureus isolates that were positive for *tst* were tested further for the production of TSST-1 using the TST-RPLA kit (Oxoid) and for enterotoxin production using the SET-RPLA kit (Oxoid) according to the manufacturer's instructions.

Phage typing. The IPS was used, which consists of 23 bacteriophages for typing of human strains of *S. aureus*. The phages were

Abbreviations: IPS, international phage set; MRSA, methicillin-resistant *Staphylococcus aureus*; SE, staphylococcal enterotoxins; TSS, toxic shock syndrome; TSST-1, toxic shock syndrome toxin-1.

group I: 29, 52, 52A, 79, 80; group II: 3A, 3C, 55, 71; group III: 6, 42E, 47, 53, 54, 75, 77, 83A, 84, 85; V: 94, 96; and M (miscellaneous): 81, 95. A standard procedure for phage typing was employed (Blair & Williams, 1961). All *S. aureus* strains included in the present study were phage typed with the exception of four clinical strains.

Antibiotic-susceptibility testing and β -lactamase production.

The susceptibility of the *S. aureus* strains to antimicrobial agents was measured *in vitro* using the disc-diffusion method (Bauer *et al.*, 1966). Susceptibility to vancomycin was also tested by the agar dilution method (NCCLS, 2000). Production of β -lactamase by *S. aureus* strains was determined by an iodometric method (Sykes, 1978).

RESULTS AND DISCUSSION

The *tst* gene encoding TSST-1 was detected in only three (7.5 %) of 40 Libyan *S. aureus* clinical strains and in none of the food strains. These three strains were also positive for TSST-1 using the TST-RPLA kit. Tsen *et al.* (1998) in Taiwan, also employing a PCR assay (using the TSST-1-specific primers in the present study), reported closely similar findings. They identified only three (4.8 %) of 62 strains of *S. aureus* from clinical sources as *tst*-carrying strains, but none of their food strains carried this gene.

Data concerning the status of TSS in Libya and the occurrence of TSST-1 in clinical and food strains of *S. aureus* from Libya and neighbouring countries are lacking. Since *Staphylococcus* infections are quite common in developing countries, it may be that this population has a higher antibody titre to TSST-1 than people in developed countries (Vergeront *et al.*, 1983). This may be the reason that TSS has not been reported so far from Libya or from many developing countries.

Our findings and those of others (Tsen *et al.*, 1998) indicate that production of TSST-1 from clinical sources other than TSS and from food is not a common phenomenon. This has been shown by several studies dealing with the detection of TSST-1 in *S. aureus* strains from TSS. Crass & Bergdoll (1986) examined cultures of *S. aureus* from individuals with confirmed or probable TSS. They found that 91.6 % of the isolates produced TSST-1 alone or in combination with one or more SEs.

It has been reported that co-production of SEC and TSST-1 or SEA and TSST-1 by *S. aureus* is common (Bohach *et al.*, 1989; De Boer & Chow, 1994; Johnson *et al.*, 1991). The results obtained in the present study are in line with the findings of the above-mentioned studies. All three of the clinical *S. aureus* strains from Libya that were positive for TSST-1 were also producers of SEC, but not of SEA, using the SET-RPLA kit.

Phage typing using the IPS showed that 52.5 % of *S. aureus* strains from Libya were untypeable. Al Bustan *et al.* (1996) found that 73 % of *S. aureus* strains from nasal swabs of healthy restaurant workers in Kuwait City were untypeable with standard phages.

Marples & Wieneke (1993) in the UK studied *S. aureus* strains from patients with diseases other than food poisoning. They observed strong associations between TSST-1 production and group I phage strains. In this study, TSST-1 was produced by only three clinical *S. aureus* strains (one belonged to phage group V, one to a mixed phage group and one was untypeable); therefore, no conclusions could be drawn from our findings.

Antibiotic-resistance patterns of the *S. aureus* strains isolated from clinical and food sources in Libya are shown in Table 1. Less than 50 % of Libyan clinical strains were resistant to penicillin and were β -lactamase producers. However, almost 75 % of Libyan strains originating from food were resistant to penicillin and were positive for β -lactamase. Fortunately, none of the Libyan *S. aureus* strains was resistant to methicillin or vancomycin.

Adesiyun *et al.* (1995) in Trinidad (a developing country) found that almost 75 % of their *S. aureus* strains isolated from clinical and non-clinical human sources were resistant to penicillin, but only 0.7 % were resistant to oxacillin, i.e. 0.7 % were methicillin-resistant *S. aureus* (MRSA). High rates of MRSA reported by some workers could be due to misidentification of susceptible strains of *S. aureus* as MRSA (Hoebe *et al.*, 1999). Although it appears from the results obtained in the present work that resistance to methicillin might not be a problem amongst *S. aureus* strains in Tripoli,

Table 1. Antibiotic-resistance patterns of *S. aureus* strains isolated from clinical and food sources in Tripoli, Libya

Values are numbers (percentages) of resistant strains.

Antibiotic	Clinical strains (n=40)	Food strains (n=23)
Penicillin	18 (45)	17 (73.9)
Ampicillin	18 (45)	17 (73.9)
Ampicillin/clavulanic acid	0 (0.0)	0 (0.0)
Azlocillin	18 (45)	17 (73.9)
Carbenicillin	18 (45)	17 (73.9)
Methicillin	0 (0.0)	0 (0.0)
Cefamandol	0 (0.0)	0 (0.0)
Cefalexin	0 (0.0)	0 (0.0)
Ceftriaxone	0 (0.0)	0 (0.0)
Ceftazidime	0 (0.0)	0 (0.0)
Amikacin	0 (0.0)	0 (0.0)
Tobramycin	0 (0.0)	0 (0.0)
Gentamicin	0 (0.0)	0 (0.0)
Clindamycin	0 (0.0)	0 (0.0)
Ciprofloxacin	0 (0.0)	1 (4.3)
Ofloxacin	0 (0.0)	1 (4.3)
Pefloxacin	0 (0.0)	1 (4.3)
Erythromycin	0 (0.0)	2 (8.7)
Tetracycline	7 (17.5)	6 (26.1)
Vancomycin	0 (0.0)	0 (0.0)

Table 2. Phage types of antibiotic-resistant *S. aureus* strains from Tripoli, Libya

Values are numbers (percentages). Phage group M, miscellaneous.

Source	Strains lysed by phage group:							Total
	I	II	III	V	M	Mixed	Untypeable	
Clinical	1 (5.25)	3 (15.8)	1 (5.3)	3 (15.8)	1 (5.25)	2 (10.5)	8 (42.1)	19 (52.8)
Food	0 (0.0)	4 (23.5)	2 (11.8)	0 (0.0)	1 (5.9)	0 (0.0)	10 (58.8)	17 (47.2)
Total	1 (2.7)	7 (19.4)	3 (8.3)	3 (8.3)	2 (5.6)	2 (5.6)	18 (50.0)	36

Libya, more studies are needed in this field to confirm this finding.

Several studies (Kimura *et al.*, 1992; Shimaoka *et al.*, 1996) have shown that the TSST-1 gene is more prevalent in MRSA than in methicillin-susceptible *S. aureus*. This may explain why *tst* was detected in only a small number of the strains examined in the present study.

The phage types of the antibiotic-resistant *S. aureus* strains from Tripoli are shown in Table 2. A number of investigators have found that strains of *S. aureus* lysed by group II phages are highly resistant to antimicrobial agents (Rosdahl *et al.*, 1990; Ross *et al.*, 1974). Most of the Libyan *S. aureus* strains that were resistant to antibiotics were either lysed by group II phages or were untypeable. However, the percentage of resistant strains lysed by group II phages was relatively small compared with that reported by Adesiyun *et al.* (1995). These researchers found that nearly 86 % of their *S. aureus* strains lysed by group II phages were resistant to antimicrobial agents compared with approximately 16 and 24 % of Libyan clinical and food strains, respectively. These differences could be due to the fact that < 50 % of the Libyan strains were typeable by the IPS compared with > 80 % of strains in the study by Adesiyun *et al.* (1995).

This is the first time that PCR detection of the TSST-1 gene in *S. aureus* has been reported from Libya, and further studies are needed on the occurrence of TSS in the community and the role of TSST-1-producing *S. aureus* in this disease in Libya.

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